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(54) Title: TOPICAL USE OF ANTI-MUSCARINIC AGENTS

(57) Abstract: A compound having anti-muscarinic activity, a dipole moment greater than 4D and anti-proliferation activity of at least 50% at 10 μM, e.g. glycopyrrolate, is useful for the treatment of skin conditions such as psoriasis.

TOPICAL USE OF ANTI-MUSCARINIC AGENTS

Field of the Invention

This invention relates to the treatment of topical conditions using anti-muscarinic agents.

Background of Invention

Gajewski, Pol. Tyg. Lek. 25(47) 1815-6 (1970), discloses that psoriatic skin rashes disappeared in the course of atropine therapy.

Various quaternary ammonium atropine-like drugs have been used in the treatment of hyperhydrosis, i.e. excessive sweating. They inhibit sweating but generally do not have systemic effects.

US-A-5185350 discloses substituted pyridinyl amines that are useful as topical anti-inflammatory agents for the treatment of various dermatoses.

US-A-5084281 discloses the use of cholinergic agents, in combination with a solution of sea water or a sea salt solution, for the treatment of persistent, neuropathic dermal ulcers.

WO-A-94/15623 discloses pharmaceutical compositions comprising various components, including urea and hyaurolonic acid, for the treatment of contact dermatitis and other topical conditions.

WO-A-98/00119 discloses the use of agents that affect non-neuronal acetylcholine functions, for the treatment of skin ailments. It also discloses that topically effective antagonists of muscarinic receptors, including ipratropium, are useful for the treatment of skin ailments. Various skin ailments that are disclosed include atopic dermatitis, neurodermatitis, psoriasis and cholinergic urticaria.

Summary of the Invention

According to the present invention, skin conditions are treated by the topical application of a quaternary ammonium or other compound having anti-muscarinic activity, a high dipole moment (greater than 4D) and high anti-proliferation activity (at least 50% inhibition at 10 μM). It may also have high receptor-binding activity (half-life for receptor dissociation greater than 0.11 h at M1). Topical compositions containing such compounds may also be new.

Description of the Invention

This invention is based at least in part on studies, using the assay described below, showing the inhibition of keratinocyte proliferation using anti-muscarinic agents. Such agents may be defined by their dipole moment. Dipole moment is related to drug polarity, which in turn is related to the penetration of a given drug through the upper layers of skin tissue. Ando, J. Pharm. Sci. (1984) 73(4):461-467, demonstrated a linear relationship between drug flux across the stratum corneum and dipole moment, and that drugs with a dipole moment of greater than 4.0 show limited systemic exposure due to poor passage across the skin to the circulatory system. The less polar of these agents, such as atropine (1.232D), and homatropine (1.066D), may be effective in the given assay, for the inhibition of proliferation, but have central nervous activity when applied topically. While this in some indications may be an attractive property (e.g. motion sickness), for local conditions in which the drug is applied topically, it can give rise to serious side effects that limit the use of the drug. Other anti-muscarinic agents also show efficacy in the proliferation assay. These agents may be characterised by dipole moments greater than that of atropine, e.g. scopolamine (3.946D), revatropate (4.168D), ipratropium (13.45D) and glycopyrrolate (15.10D). Agents with a high dipole moment are more suitable for topical administration to treat skin conditions. This invention therefore relates to the use of anti-muscarinic agents for the treatment of skin conditions, especially psoriasis, in which there is a low potential for systemic exposure as defined by a dipole moment greater than 4.0D, and preferably greater than 10D.

For use in this invention, suitable antiproliferative anti-muscarinic agents have a human plasma half-life of less than 3 hours. Systemic pharmacological effects characteristic of anti-muscarinic agents are caused by high sustained levels of drug in the plasma. This leads to distribution of the compound to receptors around the body. For effective therapy of a topically applied anti-muscarinic agent in proliferative conditions, a combination of antiproliferative activity (for efficacy) and low plasma half-life (to limit side effects) is necessary. Such compounds of short plasma half-life include, but are not limited to, glycopyrrolate, ipratropium and tiotropium.

Agents for use in this invention preferably also have high receptor-binding affinity. A long duration of action is extremely desirable for a topically applied drug to treat local conditions. This leads to low reapplication rates of medication, which in turn ensures

minimum disturbance to patient lifestyle, and high patient compliance. Compounds with high receptor binding affinity include glycopyrrolate, ipratropium and tiotropium.

Although ipratropium meets the criteria described above, it is not as satisfactory as a treatment of skin conditions when compared to glycopyrrolate and tiotropium. This is not due to its receptor affinity (which is similar to that of glycopyrrolate) but is due to its high off rate of receptor binding. Both glycopyrrolate and tiotropium have receptor off rates that are very attractive for dermal dosing. Barnes, British Journal of Pharmacology (1999) 127:413-420, showed a t ½ off set for glycopyrrolate of 96minutes compared to 59min for ipratropium in a clinical study of muscarinic activity in human smooth muscle. This attractive off rate can be defined using a tritated [N-methyl-3H]-scopolamine (NMS) assay; in this experiment, Barnes showed a 60% protection against [3H]-NMS binding at 30nM) when compared to ipratropium bromide.

At the clinical level, glycopyrrolate is known to have a longer duration of action in muscarinic antagonism than ipratropium; see J. Allergy Clin. Immunol. (1988) 82:115. In addition, in Frey's syndrome, a two day duration of action from a single dermal application appears to be common, in the use of glycopyrrolate.

In addition, Disse *et al*, Life Sciences (1993) 52/5-6:537-544, compared the dissociation rates of ipratropium and tiotropium. For muscarinic receptor M1, the half lives were 0.11 h and 14.6 h; for M3, they were 0.26 h and 34.7 h, respectively. The relatively low off rate and long half life for tiotropium are responsible for its very long duration of action in smooth muscle relaxation involving muscarinic antagonism.

More particularly, suitable agents for use in the invention may initially be identified by the Assay Protocol described below. This is a model of psoriasis and thus of a proliferative skin condition. An agent for use in the invention preferably has an IC_{50} value below 100 μM, most preferably below 10 μM, e.g. below 1 μM, and most preferably below 100 nM.

Examples of agents that can be used in the invention, provided that they meet the essential criteria, include ambutonium, benzilonium, dibutoline, diphemanil, emepronium, glycopyrrolate, isopropamide, lachesine, mepenzolate, methantheline, oxyphenonium, oxytroprium, penthienate, phenthimentonium, pipenzolate, poldine, tiemonium, tiotropium, tricyclamol and tridihexethyl. Glycopyrrolate is preferred.

These and other compounds for use in the invention may be provided in the form of a free base or salt. All such forms are within the scope of the invention, and in particular salts, organic and inorganic, are included. For example, quaternary ammonium compounds may be provided as a halide or other salt.

Many anti-muscarinic agents exhibit isomerism, whether optical or structural (stereoisomerism/regioisomerism). These include glycopyrrolate and tiotropium. Application of a single isomer or a non-stoichiometric mixture of isomers, e.g. non-racemic mixture, in the case of optical isomers, may optimise the desired antiproliferative activity.

Conventional topical formulations and administration techniques may be used. For example, suitable compositions include, but are not limited to, creams, ointments, gels, shampoos, lotions, ionotophoresis, patches and emollients. This invention also includes the use of anti-muscarinic agents to treat skin condition by topical administration, in which the drug is placed in a formulation system in which the drug flux across the skin is maintained at such a rate that systemic blood levels are retained at a low level. However, the drug flux is maintained at a level to effect topical activity in the skin. In this way, anti-muscarinic agents may be used that would otherwise be limited by their side-effects.

Conditions that may be treated include all forms of psoriasis, including psoriatic and scalp arthritis, skin cancer, melanoma, pemphigus, atopic dermatitis, neuro-dermatitis, eczema, contact dermatitis, acne, leprosy, seborrheic dermatitis, lupus and urticaria. The invention is particularly suited to the treatment of topical proliferative conditions such as psoriasis. Treatment may be combined with radiological therapy. Alternatively or in addition, treatment may be combined with a conventional agent, of which examples include steroids, vitamins A, D and their analogues, salicylates, anthralines and coal tar preparations.

The amount of the active agent to be used will depend on the usual factors, such as the potency of the agent, the nature and state of the condition to be treated, the state of the patient, etc. All these factors can be taken into account, and the relevant dose determined accordingly, by the skilled man.

Human Keratinocyte Assay Protocol

Neo-natal human epithelial keratinocytes (Biowhittaker) are grown in defined media (Keratinocyte growth medium KGM-2, Biowhittaker) until confluent. Passages 2-4

are preferred. Cells are plated in a 96-well plate at a density of 1 x 10^4 / well in 100µl KGM-2. Cells are left to settle at 37°C for 48 hours. Medium is removed and drug is added. Vitamin D3 is included as a standard, and a dose-response to the drug is performed.

Cell proliferation is measured 5 days (or 3 days in the case of ipratropium) after addition of drug. This is performed using a protein-based colorimetric assay, SRB (Sulforhodamine Blue) and read at an absorbency of 515nm. Results (tabulated below) are presented as % inhibition of control growth (no drug); it will be appreciated that glycopyrrolate is 3 orders of magnitude more active than other drugs tested, and particularly suitable for use as an anti-proliferative agent.

Atropine	30%	(10 µM)
Scopolamine	20%	(10 µM)
Propentheline	35%	(10 µM)
Glycopyrrolate	50%	(10 nM)
Vitamin D_3	40-80%	(10 µM)
Ipratropium	20%	(10 µM)

CLAIMS

1. Use of a compound having anti-muscarinic activity, a dipole moment greater than 4D and anti-proliferation activity of at least 50% at 10 μM, for the manufacture of a topical medicament for use in the treatment of a skin condition.

2. Use according to claim 1, wherein the compound has a t½ off set greater than ipatropium at 30 nM.

3. Use according to claim 1 or claim 2, wherein the compound exhibits a half-life for receptor dissociation at M1 or M3 greater than for ipratropium.

4. Use according to any preceding claim, wherein the compound exhibits IC_{50} of less than 1 μM, in the Assay Protocol described herein.

5. Use according to any preceding claim, wherein the compound exhibits one or more of the characteristics given in any preceding claim, at a level at least 80% of that for glycopyrrolate or tiotropium.

6. Use according to any preceding claim, wherein the compound is a quaternary ammonium compound.

7. Use according to any preceding claim, wherein the compound is selected from ambutonium, benzilonium, dibutoline, diphemanil, emepronium, glycopyrrolate, isopropamide, lachesine, mepenzolate, methantheline, oxyphenonium, penthienate, phenthimentonium, pipenzolate, poldine, tiemonium, tricyclamol and tridihexethyl.

8. Use according to any preceding claim, wherein the compound exists in more than one isomeric form and is used in the form of a single isomer or non-stoichiometric mixture of isomers.

9. Use according to claim 7 or claim 8, wherein the compound is glycopyrrolate.

10. Use according to claim 9, wherein the compound is SS, RR, RS or SR glycopyrrolate.

11. Use according to claim 5, wherein the compound is oxytropium.

12. Use according to claim 5, wherein the compound is tiotropium.

13. Use according to claim 12, wherein the compound is an isomeric form of tiotropium.

14. Use according to any preceding claim, wherein the condition is a topical proliferative condition.

15. Use according to any preceding claim, wherein the condition is selected from psoriasis, atopic dermatitis, neuro-dermatitis, eczema, contact dermatitis, acne, leprosy, seborrheic dermatitis, lupus and urticaria.

16. Use according to any of claims 1 to 14, wherein the condition is a skin cancer, melanoma, scalp psoriasis, psoriatic arthritis or pemphigus.

17. Use according to any preceding claim, wherein the medicament is a slow-release formulation.

18. Use according to any preceding claim, wherein the medicament is in the form of a cream, ointment, gel, lotion, patch or emollient.

19. Use according to any of claims 1 to 16, wherein the medicament is a shampoo.

20. Use according to any preceding claim, wherein the treatment additionally comprises the use of a compound selected from steroids, vitamins A, D and their analogues, salicylates, anthralines and coal tar preparations.